"PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON LEAVES OF *BUTEA MONOSPERMA (L) TOUB"*

DISSERTATION

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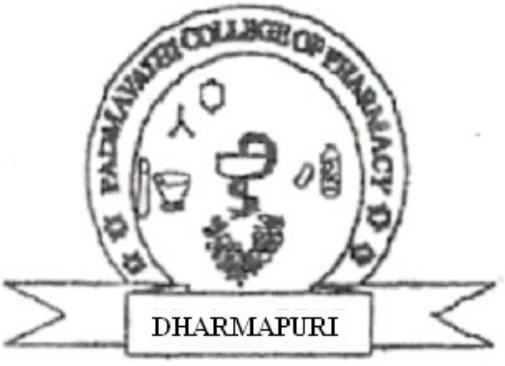

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DEPARTMENT OF PHARMACOGNOSY

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CERTIFICATE

This is to certify that this dissertation entitled **"Pharmacognostical, Phytochemical and Phramacological studies on leaves of *Butea monosperma (L) Toub"*** constitutes the original work carried out by Mr. DHIRAJ ARORA, B. Pharm. under the guidance and Supervision of Mr. L. SAMUEL JOSHUA, M.Pharm. Department of Pharmacognosy, Padmavathi College of Pharmacy, Periyanahalli, Dharmapuri- 635205.

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Dedicated

To

My Guruji

and

Respected parents

for their uncompromising

Principles that guides our life

My loving Sisters

For leading their Sisterhood into intellectual pursuits

My Hon'ble teachers for all that I am today and shall be through my life

My beloved friends

For always extending a helping hand

And

My Profession

LIST OF ABBREVIATIONS

T.S. : Transverse Section

LD_{50} : Lethal Dose 50

TLS : Tangential Longitudinal Section

RLS : Radial Longitudinal Section

TLC : Thin Layer Chromatography

HPTLC : High Performance Thin Layer Chromatography

OECD : Organization for Economic Co-operation and Development

FDP : Fixed Dose Procedure

SE : Standard Error

w/w : Weight by Weight

w/v : Weight by Volume

INDEX

1. INTRODUCTION

Planet Earth has more than 50 million species of plants, animals and micro-organism and hardly two million of them have been described by man so far. Out of 4,80,000 species of plants only 3,22,311 have been identified. India is the one of 12 mega biodiversity of world accounts for 7-8 % of recorded species of world. Over 47,000 species of plants have been invented in India and National Bureau of plant genetic resources has a collection of over 1,59,080 varieties.[1]

As per an estimate 80,000 species out of nearly 2,50,000 globally occurring species bear medicinal and aromatic value.

Out of presently available species of plants, 75 major species of medicinal flora have been highlighted and 25 out of these are most sought after during the last decade.[2]

India is the most important medicinal plant resources collection centers of the world. Over 500 million people receive the benefits of traditional knowledge of well documented and standardized systems of medicines including Sidha, Unani and Ayurveda, etc.

India has 4,60,000 practitioners of traditional system of medicines in India, as per an estimate, out of this 2,71,000 are registered and 7,843 licensed manufacturer of traditional drugs.[6]

In a review of data between 1994-1998, it was found that there is a growing interest in correlating phytochemical constituents of a plant with its pharmacological activity.[3]

Utilization of technological advances, a logical interpretation of the codified language of traditional medicine also becomes a necessity in order

to further promote research in field. ‘Rasayana’ is a good example of this, as it is attempted to interpret the word 'Rasayana' in modern scientific terminology and taking into consideration the advocated uses for this group of plants as per Ayurvedic textbooks while designing their research protocol.

However limitations in research are -

- Inadequate information on procedures adopted for quality assurance is not provided in majority of articles.
- Research areas like tuberculosis, malaria, diarrhoea, AIDS, and malnutrition which are major problems of our country are not extensively explored.
- Only few drug at experimental stages, were undertaken for clinical trial.
- Studies regarding drug interaction, adverse drug reaction are sparse.[3]

Archaeological evidence reveals that drug taking is an extremely old human phenomenon and by necessity, the drugs used in ancient civilization were extracts of plants or animal products. However, the use of discovery of medicinal property of plant parts, animal parts or of mineral origin was by chance or by hit and trial, this knowledge at one hand was transferred from generation to generation and constitutes folklore medicinal or home remedies. The same knowledge, on other hand was utilized, tried and reported by ancient physicians in their manuscripts.[6]

Ayurvedic which means science of life is Upveda of Atharva Veda,one out of four Vedas written by Aryans. Charak Samhita (1900 BC) is the first recorded treatise fully devoted to the concepts of practice of Ayurveda, has description of 341 plants and plant product for use in medicine. The next landmark of Ayurvedic literature was Sushruta Samhita (600 BC), which has

special emphasis on surgery, described 395 medicinal plants, 57 drugs of animal origin, 64 minerals and metals as therapeutic agents.[6]

Around 17th century AD, Bagbahtta of Sind, wrote Astanga Hridya, on principles and practice of medicine.

Madhab Nidana (800-900 AD) is another milestone describing diagnosis of diseases as per Ayurvedic concept, Bhava Mishra in 1550 wrote Bhava Prakasha with description of 470 medicinal plants.

Ayurveda, an ancient Indian system of health care involving holistic view of man, his health and illness and treatment consists of Salubrious use of drugs, diets and certain practices. Medicinal preparations are invariably complex mixtures, based on plant products. Around 1250 plants are currently used in various Ayurvedic preparations. Many Indian medicinal plants have come under scientific scrutiny since the middle of nineteenth century, although in a sporadic fashion. The first significant contribution from Ayurvedic Materia Medica came with the isolation of hypertensive alkaloid reserpine from the Sarpagandha plant (*Rauwolfia serpentina*), valued in Ayurveda for the treatment of hypertension, insomnia and insanity. This was the first important ancient-modern concordance in Ayurvedic plants.[6]

With advancement in chemistry and biology, disciplines central to the study of biological activities of natural products, many Ayurvedic plants have been reinvestigated.

In the early development of modern medicine, biologically active compounds from higher plants have played a vital role in providing medicines to combat pain and diseases. In British Pharmacopoeia of 1932, over 70% of organic monographs were on plant derived products.

However with the advent of synthetic medicinal and antibiotics, the role of plant derived therapeutic agents significantly declined, thus in 1980 edition of British Pharmacopoeia, the share of plant-based monographs fell to approximately 20% and out of new chemical entities introduced as medicinal agents the share of plant-based drugs was not more than 2%..[6]

The practical aspect of using synthetic drugs was that only one molecule out of the 1000 synthesized came into usage for patients, after incurring a heavy Research and development expenditures, with lots of investment in terms of time and labour.

In spite of these, withdrawal rate of new synthetics has been high, may be because of long-term side effects, safety data, emergence of resistance and many other reasons. This phenomenon has led researchers back into the field of plant-based drugs. Now Investigations and use of plant based material is on single plant extracts or fractions thereof. These new plant derived products, if need be, are used as lead structures also, are carefully standardized and their efficacy and safety for a specific application have been demonstrated. Thus plant based therapeutic agents continue to have significance and appear to be gathering momentum in health related areas.[10]

With advancement of technology, now it is possible to approach the study of medicinal plants from the botanical, phytochemical and pharmacological point of view. The use of modern isolation technology and pharmacological testing procedures means that new plant drugs usually find their way into medicine as purified substances rather than in the form of Galenical preparation.[5]

Plant Kingdom still holds many species of plants containing substances of medicinal value, which have yet to be discovered, large

numbers of plants are constantly being screened for their possible pharmacological value. The wealth of uninvestigated plant material available can be illustrated by the fact than in 1985, out of 3500 new chemical structures from natural products, 2600 were from higher plants.

Mentioned below are some drugs of natural origin, which have become drug over choice because of their efficacy and safety over currently available drugs:

- Mevastatin (compactin) isolated from Penicillin sp., and lovastatin and simvastatin.
- Qunine- still preferred drug against P. falciparum
- Penicillin, Cephalosporins, Vancomycins
- Artemisinin and its derivatives-. Artisuanate, Artemether, Arteether and Sodium.
- Immunosuppressants - Cyclosporin and Rapamycin
- Prolactin secretion inhibitor - Ergoline, Terguride
- Anticancer - Vinblastine, Vircristine, Etoposide, Teniposide, Paclitaxel, Topotecan[4,5]

All research programs that aim to discover novel bioactive compounds from natural sources relay to some degree on the assumption that a carefully reasoned philosophy guiding the selection of species to be sampled can improve the rate of discovery. The geographic and taxonomic distribution of 2,50,000 species of higher plant is not random, the chemistry of various plant families is known to differ significantly and our present state of knowledge of chemistry of plants remain uneven.

Collection for screening may be random or guided by Taxonomy, Phytochemistry, Ethanobotany, or Zoopharmacology (how certain animals utilize plants).

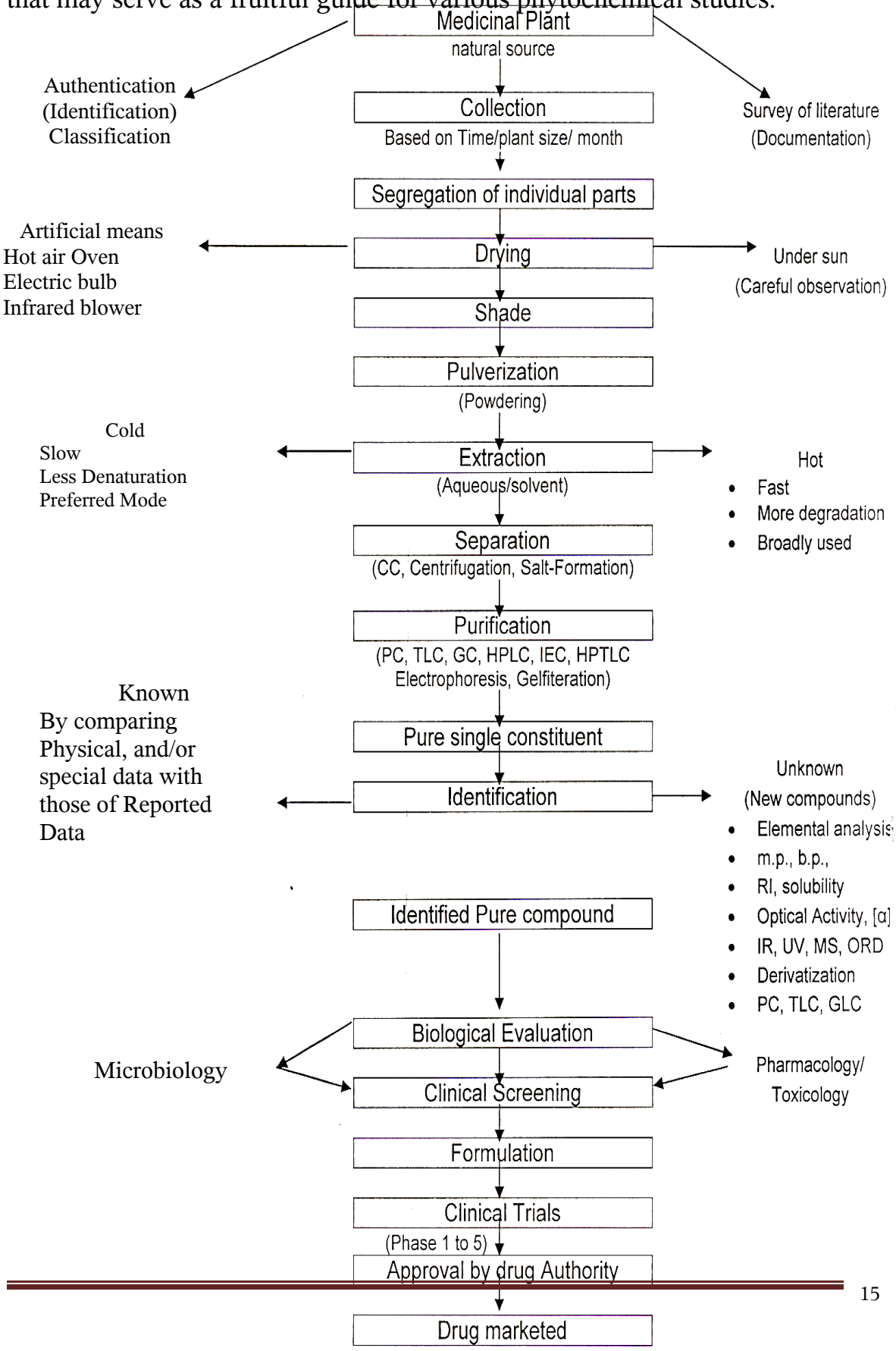
It is observed that Ethanobotanically guided collections has yielded higher rates than other sampling strategy but has two problems, first they are based on relatively small set of data and results though suggestive, are not statistically significant, and secondly, they are based on analysis of rates of positive results in primary screen.[5,7]

In general, natural drug substances offer four vital and appreciable role in the modern system of medicine thereby adequately justifying their legitimate presence in the prevailing therapeutic arsenal namely-

- Serve as extremely useful natural drugs: e.g. Atropine, Ergotamine, morphine, etc.
- Provide basic compounds affording less toxic and more effective drug molecules: Morphine (natural), hydromorphine (semisynthetic), methadone (synthetic), ephedrine (natural), phenylpropanolamine (semisynthetic), tetrahydrozoline(synthetic).
- Exploration of biologically active prototypes towards newer and better synthetic drugs : Procaine from cocaine, chloroquinine from quinine
- Modification of inactive natural products by suitable biological/chemical means into potent drug: Taxol from 10-Dasacetylbaccatin III, Progesterone and Pregnenolone from diosgenin.[10]

As per global survey by World Health Organization (WHO), around 20,000 medicinal plants are being used profusely either in Pharmaceutical industry or in folk medicine. Interestingly about 1.4% does possess well-established widely unequivocally active constituents. The figure on the next

page illustrates schematic development of a drug from a "medicinal plant" that may serve as a fruitful guide for various phytochemical studies.[9]



2. LITERATURE REVIEW

Systemic literature review is the basis for planning a scientific work. In this section review of literature of *Butea monosperma (L) Toub* under various heading have been carried out. They are Pharmacognostical review, Phytochemical review, Pharmacological review, Ethanomedical review and miscellaneous review. Some of the pioneering work in this field is given below.

2.1 Pharmacognostical Review:

- Burli DA and Khada A (2007)**:-** A comprehensive pharmacognostical review on *Butea monosperma (Lam.) Kuntze.*[11]
- Shome U, Khanna RK et al (1980):- Pharmacognostical characters of *Butea monosperma* flowers including epidermal and anatomical characters of the flower parts, phytochemical tests, fluorescence tests and TLC of different extractive have been reported.[12]
- Laghate PV, Grampurohit et al (2003):- Reported pharmacognostical and phytochemical investigation on the roots of *Butea monosperma (Lam.) Taubz.*[13]

2.2 Phytochemical Review:

- Jain SC, Kamal R et al (1980)**:-** Identified by co- TLC in various solvents and other spectral properties.[14]
- Yadav AS, Mishra GP (1983):- Studied color & pH of seeds / fruit leachate of *Butea monosperma.*[15]
- Wagner H, Geyer B et al (1986)**:-** Reported qualitative and

quantitative analysis of isobutrin and butein in extract of *Butea monosperma* flowers.[16]

- Porwal M, Sharma S et al (1988):- Isolated & Identification of a new derivative of allophonic acid as 2-hydroxy-omega methyl allophonic acid (as methyl ester) from the seed coat of *Butea monosperma* (*Lam) Kuntze.*[17]
- Mishra, Shukla YN et al (2000):- Isolated two new compounds from the stems of *Butea monosperma* have been characterized as 3-alpha hydroxyeuph-25-ene and 2,14-dihydroxy-11,12-dimethoxy-octadec II-encyclohexane by spectral data and chemical studies.[18]
- Mishra, Shukla YN et al (2000):- Identified flavanoids from methanolic extract of *Butea monosperma* flowers as butrin, butein, 3,4,7-trihydroxy flavone and stigmasterol-3 beta-D-glucopyranoside.[19]
- Shukla YN, Mishra M (2000):- Identified two novel compounds from *Butea monosperma* leaves as 3,9-dimethoxypterocaopan and 3-alpha-hydroxyeuph-25-enylhepta-cosanoate.[20]
- Shukla YN, Mishra M (2002)**:-** Identified & Isolated pterocarpan, phenol and lipid constituents from *Butea monosperma* stem.[21]
- Shukla YN, Mishra M (2000):**-** Isolated & Identified Euphane triterpenoid ester and pterocarpan from *Butea monosperma* leaves.[22]

2.3 Pharmacological Review:

- Gunakkunru K, Padmanaban et al (2005)**:-** Reported anti-diarrhoeal potential of the ethanolic extract of stem bark of *Butea monosperma (Lam) Huntz* has been evaluated using several experimental models in wistar albino rats.[23]

- Miriyala Sumitra, Panchatcharam Manikandan et al (2005):- have reported efficacy of *Butea monosperma* on dermal would healing in rats.[24]
- Veena S, Kasture SB et al (2002):- Reported anticonvulsive activity of *Butea monosperma* flowers in laboratories animals.[25]
- Zaqar Iqbal, Muhammad lateef et al (2006):- have reported In vivo anthelmintic activity of *Butea monosperma* against trichostrongylid nematodes in sheep.[26]
- J.H. Bavarva, Narasimhacharya (2008):- have reported preliminary study on antihyperglycemic and antihyperlipidemic effects of *Butea monosperma* in NIDDM rats.[27]
- Shahavi VM, Desai SK (2008):- have reported anti-inflammatory activity of *Butea monosperma* flowers, studied methanolic extract of *Butea monosperma* flowers (MEBM) for anti inflammatory activity against carageenan induced paw edema and cotton pellet granuloma in albino rats.[28]
- Prashanth D, Asha MK et al (2001):- have reported that methanol extract of *Butea monosperma* seeds, tested in vitro showed, significant anthelmintic activity.[29]
- Bhatwadekar AD, Chintawar SD et al (1999):- have reported Antistress activity of *Butea monosperma* flowers.[30]
- Chauhan S, Mathur R. (1995):- reported Antifertility activity of ethanolic extract of *Butea monosperma* flowers in male rats.[31]
- Gawal NS, Pal SC et al (2007):- studied effect of extract of dried flowers of *Butea monosperma* on memory and behavior mediated via monoamino neurotransmitters in laboratory animals.[32]
- Prajapati ML, Sharma AK et al (2000):- Conducted clinical evaluation

of palasha pushpadi churna, (*Butea monosperma* flowers powder) in the management of Diabetes mellitus.[33]

2.4 Ethnomedicinal review:

- Baillore KV, Audichya KC (1978):- was observed during a field survey in tribal areas of Rajasthan and Gujarat that *Butea monosperma* was being used as contraceptive drug.[34]
- Garg SK, Mathur VS et al (1978):- have reported antifertility activity of seeds of *Butea monosperma.*[35]
- Shaw BP,Tripathi AK. (1982):- Reported anthelmintic activity of crude powdered seeds of *Butea monosperma.* [36]
- Phadke UR,Ghooi RB (1983):- Reported Sodium salts of the phenolic constituents from the bark of *Butea monosperma* show antiasthmatic activity.[37]
- Mehta BK, Dubey A (1983):- Observed significant antifertility activity in the alcoholic extract of the outer seed coat and seed powder of *Butea monosperma.*[38]
- Ansari NA, Rastogi SK et al (1984):- reported Preliminary study of aqueous extract of *Butea monosperma* Seeds for anthelmintic activity.[39]
- Agarwal AK, Singh M et al (1994):- Reported that pipali rasayana an Ayurvedic herbal medicine, prepared from *Butea monosperma* Prescribed for the treatment of chronic dysentery and warm infection was tested for anti-giardial and immuno stimulatory activity in mice, infected with Giardia lamblia trophozoites.[40]
- Choudhary RR (1995):- Reported regulation of fertility by plant

product – theme talk of *Butea monosperma.*[41]

- Basu R, Mukherjee PK (1999):- Reported that *Butea monosperma* used for lac culture by the tribals of Purulia in West Bengal.[42]
- Agarwal AK (2000) :- developed Ayurvedic preparation by giving bhavna to piper longum fruit powder with Ash (Bhasma) of *Butea monosperma* resulted into remarkable potentiation of antigiardiac effect.[43]
- Kumar KV, Malhotra D et al (2001):- Conducted survey on ethno-medico-botanical of *Butea monosperma* used by the tribals of sehore district of M.P. for curing various ailments.[44]
- Pandey HP (2001):- Reported that seed oil of *Butea monosperma* used as a traditional several toner and contraceptive.[45]

2.5 Miscellaneous review:

- Verma R (1982):- *Butea monosperma* has been discussed under the following headings: local names, phytography botany, phytochemistry and phytopharmacology.[46]
- Prasad UN (1982):- *Butea monosperma* tree with pale-yellow flowers has been reported for the first time.[47]
- Khare PK, Mishra GP (1981):- Observed relative growth performance of seedling of *Butea monosperma (Lam) Taub* in three artificial soil types.[48]
- Jain S, Joshi IJ et al (1983):- Reported cingulata (A New leaf spot disease) on *Butea monosperma.*[49]
- Purohit M, Jamaluddin (1988):- Found Twin and tricotyledonous seedling (Abnormal seedling) of *Butea monosperma*.[50]

- Kulkarni N, Joshi KC (1995):- First report of spodoptera litura (Fab) boursin (Lepidoptera: Noctuidae) as a pest on *Butea monosperma (L) Toub*.[51]
- Upadhya AS, Kumbhojkar MS (1997):- Observed during the medico botanical survey that tribal communities use leaves of *Butea monosperma* during worship.[52]
- Verma M, Shukla YN (1998):- Provided a review on the chemistry, biological activities, biology, cultivation and use of *Butea monosperma*.[53]
- Purohit M, Jamaluddin et al (1998):- Studied germination percentage, seed borne fungi and effect of different fungicides on seed borne fungi of freshly collected seeds of *Butea monosperma*.[54]
- Gupta S, Khan R (2003):- Developed the Nursery techniques of *Butea monosperma* plants in vidhyan and central plains of U.P.[55]

3. RESEARCH ENVISAGED

3.1 Aim of present study:

In recent years there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results. Due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic complications.

As we know that everything in this world change time by time, since thousands of year the era was of Ayurveda or herbal origin drug. But last few decades it was replaced by allopathic system of medicine, which was rapidly accepted world wide, but latter due to its lots of adverse effect, again men step down on Ayurveda because of its better therapeutic result and safety profile and now the people are more believing in natural origin drug.

Numerous drugs have been entered in the international pharmacopoeia through the study of ethanopharmacology and traditional medicines. For Ayurveda and other traditional medicines newer guidelines of standardization, manufacture and quality control are required. Employing a unique holistic approach, Ayurvedic medicines are usually customized to an individual constitution. Traditional knowledge driven drug development can follow a reverse pharmacology path and reduce time and cost of development. Powerful new technologies, such as automated separation techniques, high throughput screening and combination chemistry are revolutionizing drug discovery. Traditional knowledge will serve as a powerful search engine and most importantly, will greatly facilitate

international focused and safe natural product research to rediscover the drug discovery process.

Looking to the scope of herbal drug and increasing demand especially in disease of liver, hypertension, diabetes, cancer, renal diseases, inflammation, infectious diseases, arthritis and skin disease etc., hence, it is planned here to study the plant like *Butea monosperma (L) Toub* The selection of the plant *Butea monosperma (L) Toub* was made on the basis of its

- Easy availability
- Therapeutic value
- Degree of research work which is not done
- Useful chemical constitute present

Keeping in mind about the adverse effects of allopathic drugs like anti diabetics, anti pyretic and anti-inflammatory, above said activity were studied with different parameters in order to give possible scientific validation.

3.2 Plant Collection and Identification:

The plant *Butea monosperma (L) Toub* belonging to family "Fabaceae" are widely available in Tamilnadu. For present work the plant *Butea monosperma (L) Toub.* was collected in the month of July 2008, from Orakadam forest near Chennai.

Taxonomical Identification:

The plant was identified by Dr P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai by specimen no. PARC/2008/241.

Treatment :

The leaves were collected and washed with water and dried. It in sunlight one hour and then it was dried in shade. The aerial pieces were powdered by means of a wood-grinder and the powder was passed through the sieve no.60 for powder analysis and the coarse fraction was subjected for phytochemical studies.

3.3 Plan of work:

The plan of work of the Leaves of *Butea monosperma (L) Toub* was carried out as follow:

1. Pharmacognostical Evaluation:

A. Macroscopical Studies.

B. Microscopical Studies.

C. Physico-chemical constants:.

a. Ash values.

b. Extractive values.

c. Loss on drying.

D. Foaming index.

E. Fluorescence analysis.

2. Preliminary Phytochemical Evaluation:

A. Purification of solvents.

B. Preparation of extracts.

C. Qualitative phytochemical analysis.

D. Chromatographic studies:

a. Thin layer Chromatography.

b. High Performance Thin Layer Chromatography.

3. Pharmacological Studies:

a. Acute oral toxicity study

b. Antidiabetic activity

c. Anti-inflammatory activity

d. Antipyretic activity

4. BOTANICAL INFORMATION

4.1 Scientific Name: *Butea monosperma (L) Toub*

4.2 Synonym: *Erythrima monosperma Lam., Butea frondosa Koen.*

4.3 Taxonomy:

Kingdom	-	*Plantae*
Subkingdom	-	*Tracheobionta*
Division	-	*Magnoliophyta*
Class	-	*Magniloipsida*
Subclass	-	*Rosidae*
Order	-	*Fabalesa*
Family	-	*Fabaceae*
Genus	-	*Butea*
Species	-	*monosperma (L) Toub.*

4.4 Vernacular Names:

Latin	-	*Butea monosperma*
Tamil	-	Parasa
English	-	Butea Gum Tree
Sanskrit	-	Palasha
Hindi	-	Dhak
Gujrati	-	Kesudo
Kannad	-	Muttuga
Oriya	-	Porasu

Telgu - Moduga

4.5 Occurrence:

Grow gregariously on open grassland and scattered in mixed forest. Plantation can be raised both on irrigating and dry lands.

4.6 Distribution:

Asia Tropical: Pakistan, Srilanka, Mayanmar, Thailand, Vietnam and in warmer parts of India.

4.7 Ethanomedical Information:

A red juice exudes from natural cracks and also from artificial incisions in the bark. The fresh juice is applied to ulcers and in relaxed, congested and septic sore throat. The gum is a powerful astringent; it is given internally for diarrhoea and dysentery, phthisis and haemorrahge from stomach and bladder, its infusion is occasionally employed as a local application in leucorrhoea. A solution of the gum is applied to bruises and erysipelatous inflammations and ringworm.

The bark is reported to posses astringent, bitter, pungent, alterative, aphrodisiac and anthelmintic properties. It is useful in tumors, bleeding piles and ulcers. The decoction is prescribed in cold, cough, fever, various forms of hemorrhages, in menstrual disorders and in the preparation of tonics and elixirs, an alcoholic extract of the bark is reported to inhibit the activity of *Escherichia coli* and *Micrococcus pyogenes* var. *aureus.* A fraction,

containing the sodium salt of phenolic constituents, isolated from the bark, has shown potential as an anti-asthmatic agent in experimental animal.

The roots are useful in elephantiasis, and in curing night blindness and other defects of sight. They are also reported to cause temporary sterility in women.

The green leaves are commonly lopped for fodder. The yield of milk in buffaloes, fed with Butea leaves, is reported to improve. They are credited with astringent, tonic, diuretic and pimples and tumorous haemorrhoids and are internally given in flatulent colic, worms and piles.

The flowers are reported to possess astringent, diuretic, depurative, aphrodisiac and tonic properties; they are used as an emmenagogue and as poultice in orchitis and to reduce swellings for bruises and sprains. They are also effective in leprosy, leucorrhoea and gout.[58]

Fig. 4.1: Exomorphic Feature of whole plant of *Butea monosperma (L) Toub*

5. PHARMACOGNOSTICAL STUDIES

5.1 Introduction:

These are the studies which deal with its morphology, Microscopical, and Physico-chemical constant of the plant material, which has the Pharmaco-therapeutic property.

5.2. Macroscopical characters: [56]

Butea monosperma is a deciduous moderate sized tree with grey bark a red gum, Kino, exudes from the bark.

WOOD:

The wood is dull white and soft.

LEAVES:

The leaves are tripinnately compound. The three leaflets are abovate, margins smooth, abaxial surface hairy, adaxial surface glabrous.

FLOWERS:

The flowers are densely fascided, flowers bright red, large and showy (fig. 1). The sepals are five gamosepalous, valvate in aestivation, petals are five, differentiated into one standard petal, two wing petals and two keel petals, aestivation unilencate. Stamens- ten stamens, diadelphous with a stamens in bundle and 10^{th} stamen being solitary, overy- monocarpellary, many ovules on marginal placentation.

SEEDS:

Seeds are elliptical, reddish brown seed coat and hard.

SHOOT:

Shoots are clothed with gray or brown silky pubescence.

5.3. Microscopical features:

5.3.1. Introduction:

Microscopy is an important tool in the evaluation of crude drugs which is applicable at various levels such as the authentification of crude drugs, study of powdered drugs, calcium oxalate crystals, starch grains, pollen grains etc. Ash values, extractive values and foaming index are used for the study of physical properties.

Arrangement of plant into groups and subgroups is commonly spoken as classification. Various systems of classifying plants have gradually developed during past few centuries, which have emerged as a discipline of botanical science known as taxonomy or systemic botany. The word 'taxonomy' is derived from two Greek words 'Taxis' meaning as arrangement and 'Nomas' meaning laws. Therefore, the systemization of our knowledge about plants in an orderly manner becomes subject matter of systematic botany.[57]

The aim and objective of taxonomy is to discover the similarities and differences in the plants, including their closed relationship with their descents from common ancestry. It is a scientific way of naming, describing and arranging the plant in an orderly manner.

5.3.2 Materials and Methods for Anatomical studies:

The Plant Care was taken to select healthy plants and for normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin – 5ml + Acetic acid – 5ml + 70% Ethyl alcohol – 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass,

1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

A. Sectioning:

The paraffin embedded specimens were sectioned with the help of Rotary Microtone. The thickness of the section was 10-12 µm. Dew axing of the sections was by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per the method published by O'Brien et al. (1964). Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., wherever necessary sections were also stained with safranin and Fast-green and IKI (for Starch) [56,59].

For studying the stomatal morphology, venation pattern and trichomes distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell component were studied and measured. [59,60]

B. Photomicrographs:

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications

were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books (Esau, 1964). [61]

5.3.4 Anatomy of Leaf:

Leaflets:

The leaflets are dorsiventral with prominently projecting lateral veins (fig. 2.1), thick midrib (fib. 3) and bilateral lamina (fig. 2.2).

The lamina has even and smooth adaxial surface and uneven abaxial. Surface (fig. 2.2). It is 170 um thick. The adaxial epidermis has rectangular or squarish cells with prominent cuticle. The cells are 20um thick. The abaxial side has narrow epidermis with cylindrical, cuticularised cells.

The mesophyll tissue is characteristic is having a wide zone of palisade cells comprising of two layers narrow cylindrical compact cells. The palisade zone is 90 um in height. Beneath the palisade zone is a single horizontal layers of rectangular hyaline parenchyma cells.

The abaxial part of the lamina consists of a single row of palisade cells. These abaxial cells are cylindrical and vertical and loosely arranged with wide air spaces. The abaxial palisade zone is 30um in height.

The lateral vein has prominent collateral vascular strand with in abaxially projecting part. The vascular strand is covered by thick

paranchymatous bundle sheath which extends adaxially into a thick vertical coloumn (fig. 2.1)

The veinlets also have prominent vascular strand and paranchymatous bundle sheath and adaxial and abaxial extensions (fig. 2.2).

Midrib:

The midrib is very thick having adaxial, broadly conical hump and more or less wide circular abaxial part. The midrib is 1.1mm thick in vertical plane. The adaxial hump is 200um in height and 450um in width. The abaxial part is 1.1mm wide. (Fig. 3)

The epidermal layers of the midrib are thin with small squarish cells and thick cuticle. the ground tissue consist of about three layers of collenchymas and four or five layers of parenchyma, same of the cells in the abaxial part are crushed forming thin dark line.

The vascular system is complex (fig. 3,6.1). It has wide hollow cylinder occupying the major area of the midrib. The vascular cylinder consists of a thick circular cylinder of sclerenchyma. The cylinder encloses a wide and deep abaxial urn-shaped bond of five vascular. Segments, a short arc of adaxial segment and two centrally placed circular vascular strands. The vascular strands have short closely arranged radial rows of wide, angular thick walled xylem elements; each segment has thick arc of phloem on the outer part.

The metaxylem elements are 30-35um wide.

Venation pattern:

The lateral veins and vein lets are thick and straight. They form narrow but distinct vein islets which are polygonal in outline and random in

orientation. Some of the islets have no vein terminations while others have short thick, straight unbranched terminations. (Fig. 4.1,2)

Epidermal Cells and Stomata:

The epidermal cells are narrow in area; their anticlinal walls are thin and wavy. The stomata are paracytic type with equal or unequal lateral subsidiary cells. The guard cells are elliptical or circular measuring 15x20 um in size. (Fig. 5.1, 2)

5.3.5 Powders Microscopy:

Epidermal trichomes are abundant on the abaxial leaf surface. The trichomes are unicellular, unbranched, narrow, and thin walled and pointed at the tip. The trichomes are glandular type (Fig. 6.2). They are up to 60um long and less than 5um thick. The cell walls are smooth.

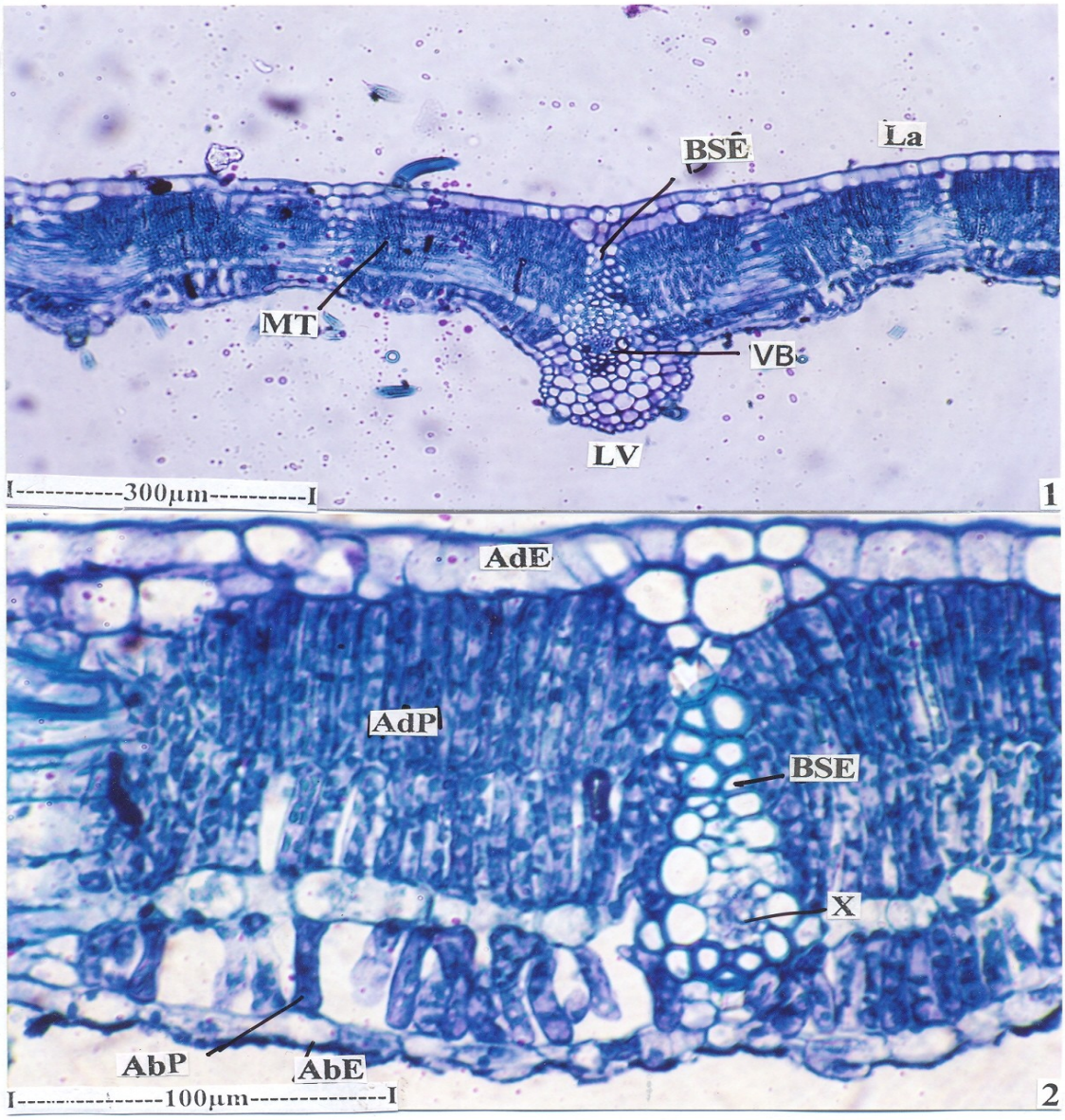
Petiole (Rachis) (fig.7.1, 2):

The petiole is circular in outline, the dorsiventral, symmetry is not evident. It has thin epidermal layers of small, thick walled cells and fairly wide outer paranchymatous ground tissue. The vascular system is complex, possessing a continuous ridged outer cylinder and central excentric group of discrete strands. The outer cylinder consist of several wedge shaped vascular segments, arranged closely appearing as continuous cylinder. A thick sclerenchyma are occurs on the outer part of each segment (fig. 7.2). The central groups of vascular strands are random in orientation and closely crowded. Each segment has its own. Phloem and sclerenchyma cap.

Fig. 1: Exomorphic features of the Leaf and Flowers of *Butea monosperma (L) Toub*

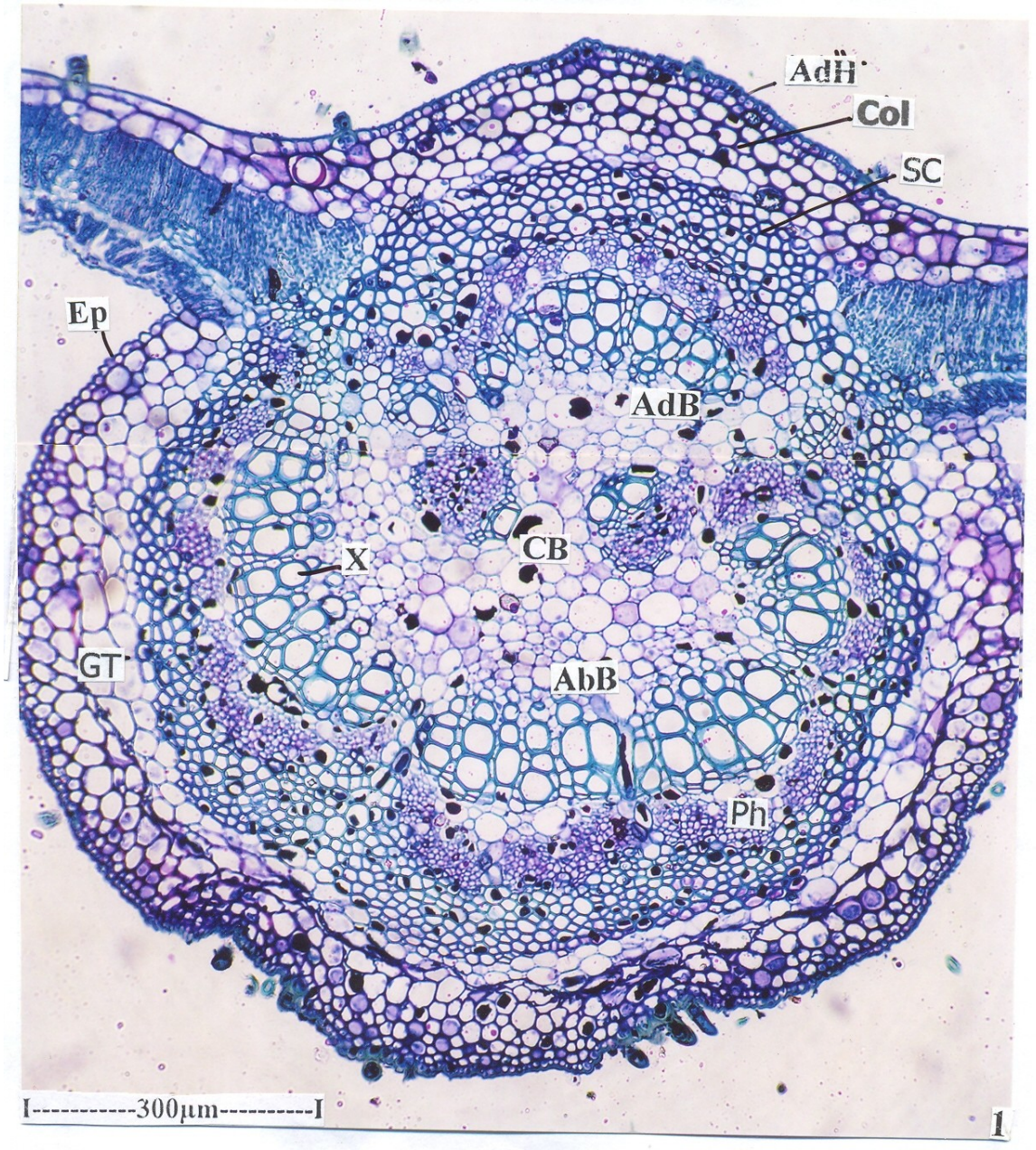
Fig. 2: Anatomy of the lamina:-

1. **T.S. of lamina through lateral vein**
2. **T.S. of lamina**



(AbE – Abaxial epidermis, AbP – Abaxial palisade, AdE – Adaxial epidermis, AdP – Adaxial palisade, BSE – Bundle sheath extension, La – Lamina, Lv – Lateral Vein, MT – Mesophyll tissue, VB – Vascular bundle, X – Xylem

Fig.3: Structure of Midrib:

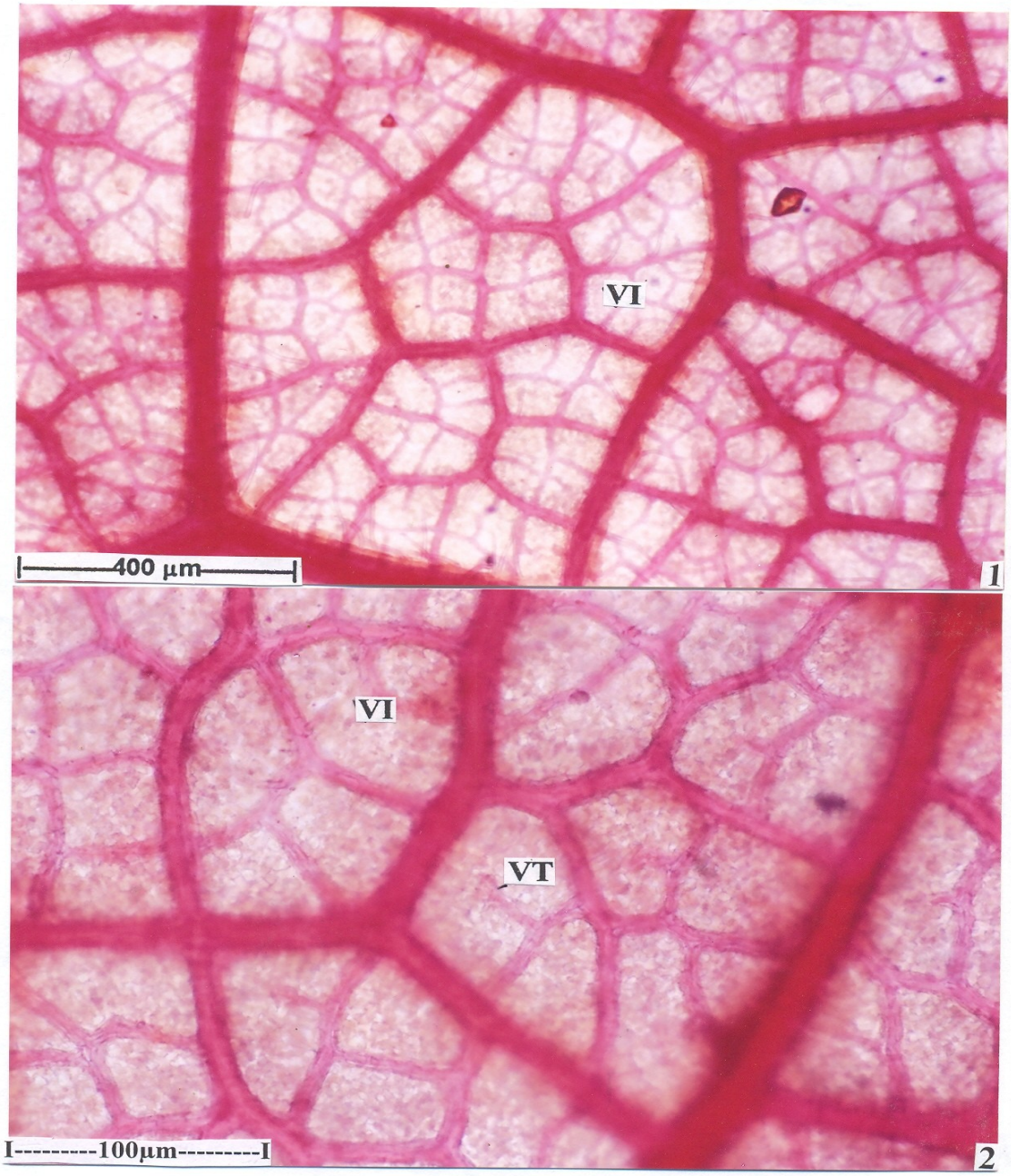


(AbB – Abaxial bundle, AdB – Adaxial bundle, AdH – Adaxial hump, CB – Central bundle, Col – Collenchyma, Ep – Epidermis, GT – Ground tissue, Ph – Phloem, Sc – Sclerenchyma, X – Xylem).

Fig. 4: Venation pattern:

(1) Cleared leaf showing vein islets and vein – termination

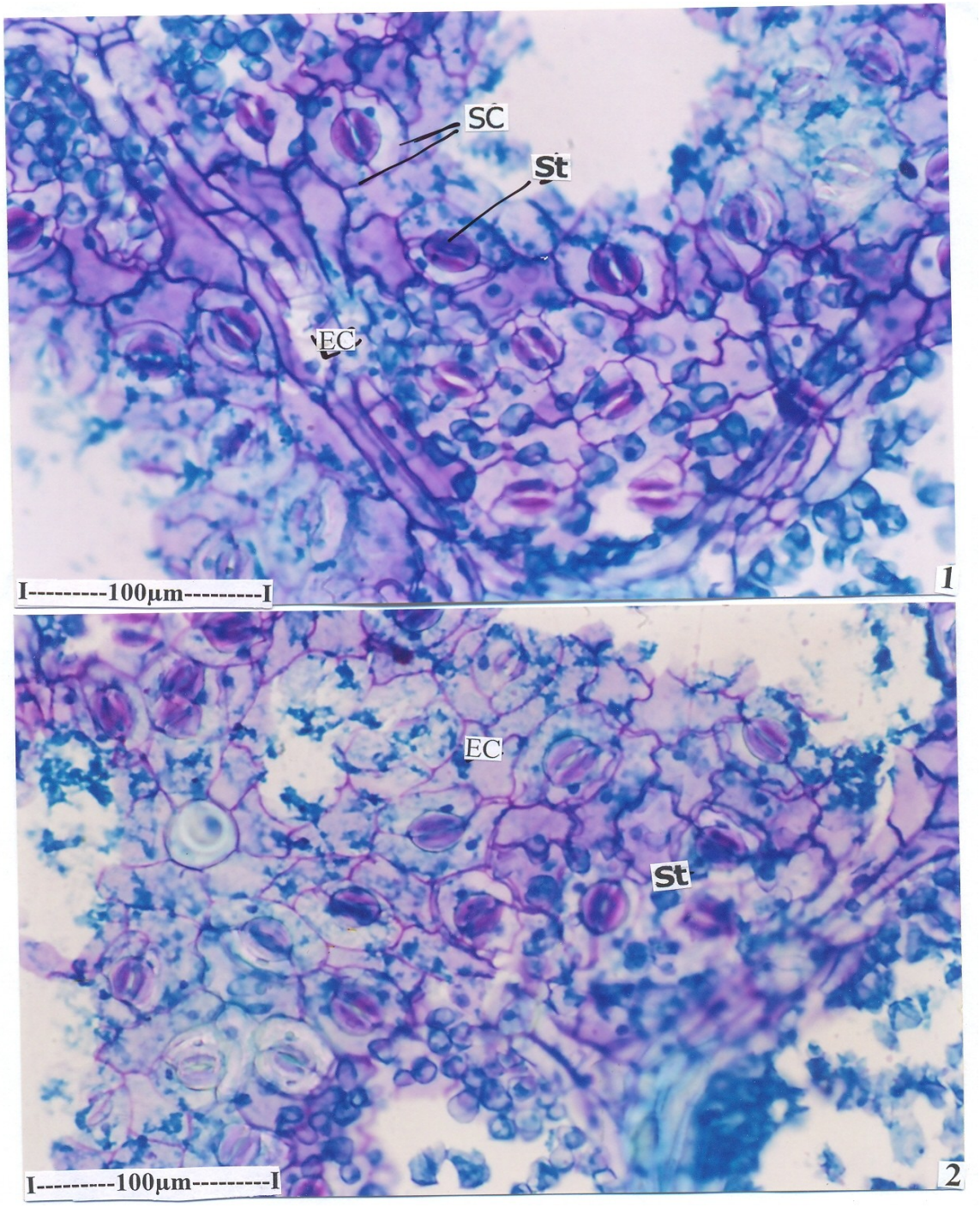
(2) **Enlarged feature showing vein islets and vein – termination**



(VI – Vein islets, VT – Vein - termination).

Fig. 5: Stomatal Morphology:

1,2. Para dermal section showing abaxial epidermis with stomata

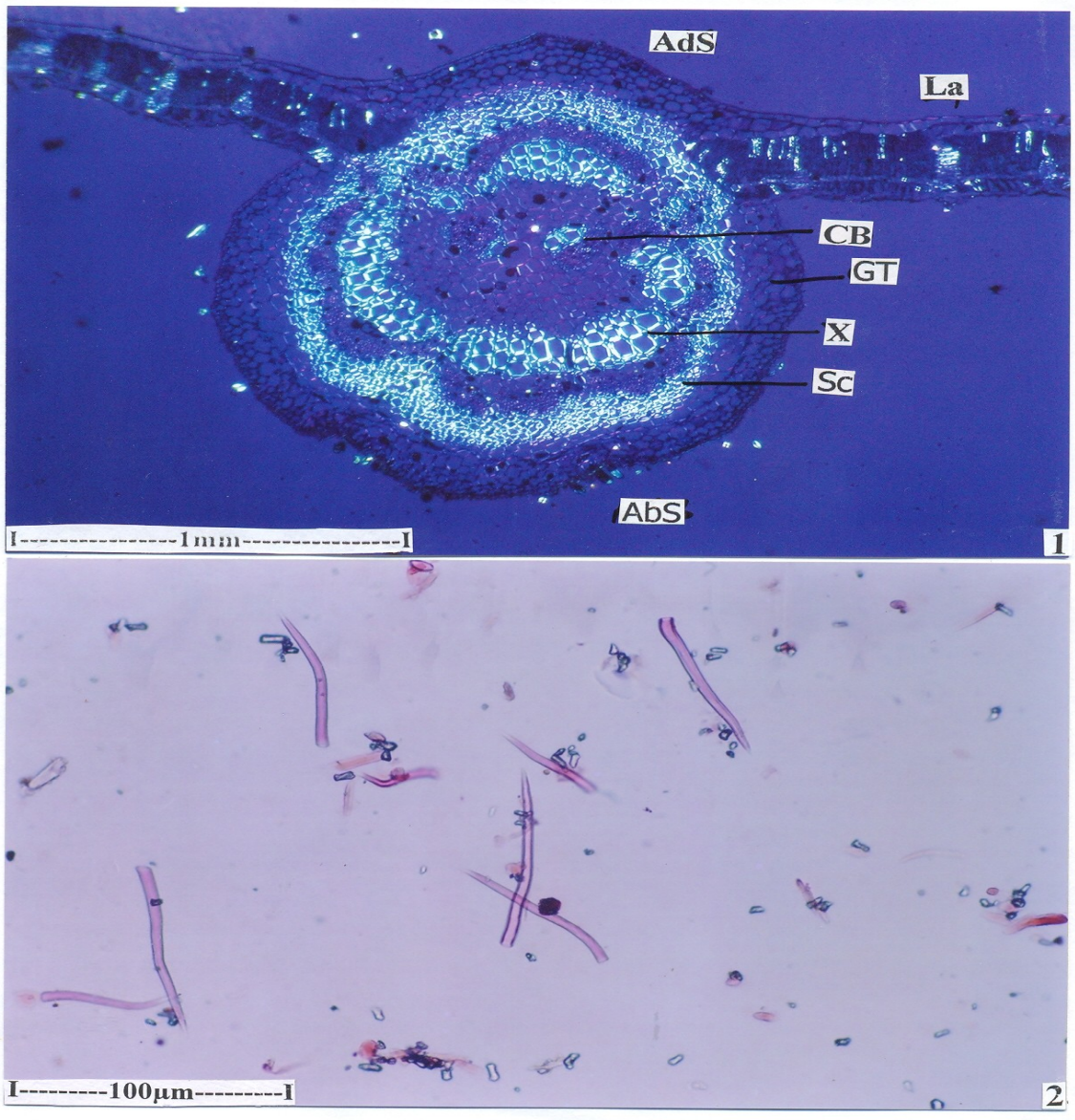


(EC – Epidermal cell, SC – Subsidiary cells, St – Stomata)

Fig. 6: Crystal distribution and powder microscopy of the leaf:

(1) T.S. of leaf showing crystals in the mesophyll tissue (under polarized light microscope).

(2) Non-glandular epidermal trichomes in the leaf powder.

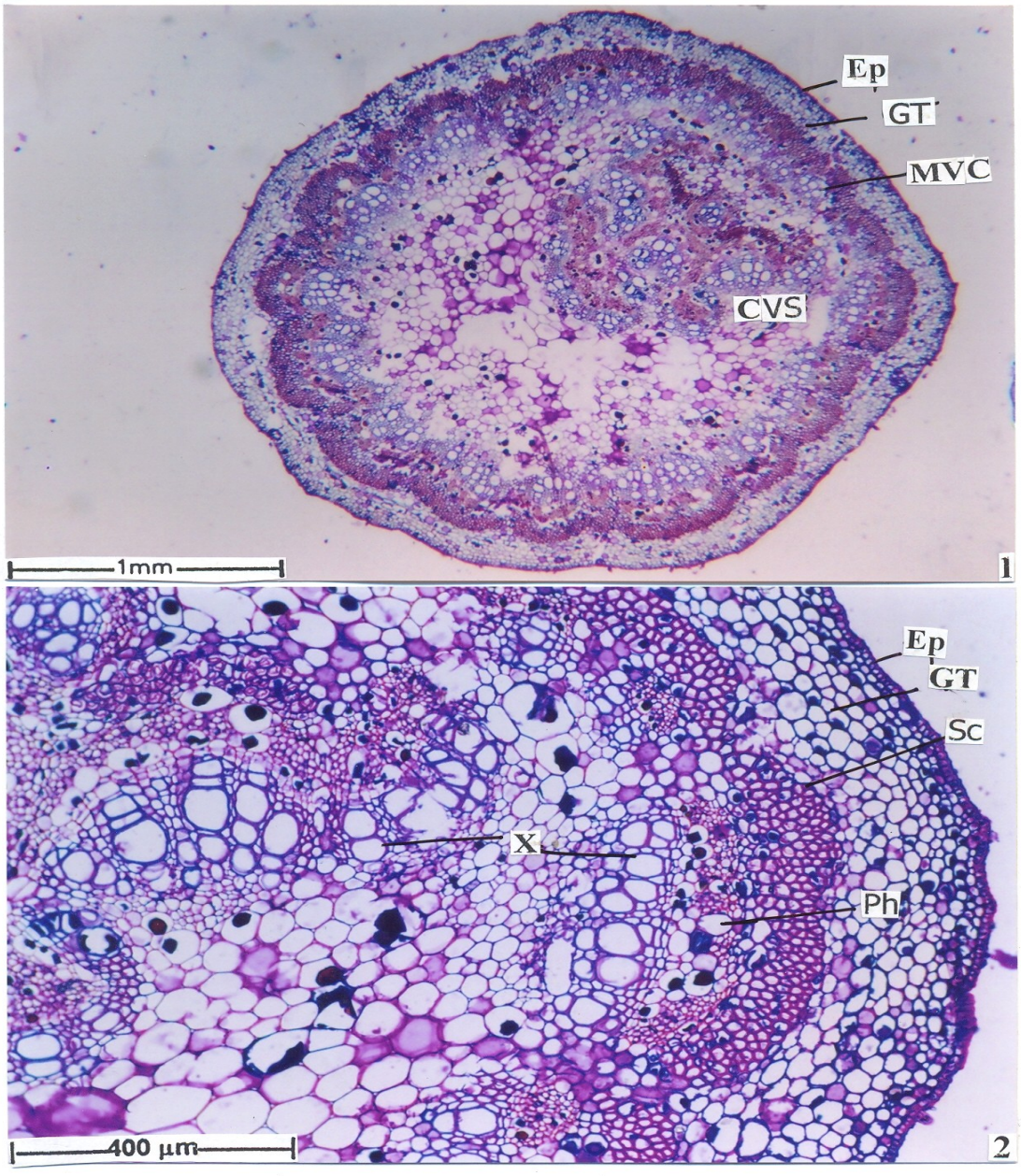


(Abs – Abaxial side, Ads – Adaxial side, CB – Central bundle, GT – Ground – tissue, La – Lamina, Sc – Sclerenchyma, X – Xylem).

Fig. 7: Anatomy of the rachis:

(1) T.S. of rachis entire view.

(2) **T.S. of rachis a sector enlarged**.



(CVS – Central vascular strand, Ep – Epidermis, GT – Ground tissue, MVS – Median vascular cylinder, Ph – Phloem, Sc – Sclerenchyma, X – Xylem

6. PHYTOCHEMICAL STUDIES

6.1 Introduction:

Plants may be considered as biosynthetic laboratories in which various kinds of organic compounds are synthesized such as Carbohydrates, Proteins, Lipids, Flavonoids, Glycosides, Alkaloids, Volatile oils, and Tannins etc., which exert a physiologic effect and are utilized as biologically active components by men since time immemorial. The medicinal value of any plant drug however depends on the nature of chemical constituents present in it and is referred to as active principle.[67]

For our present study, we had taken of *Butea Monosperma (L) Toub* to extract the active constituents are tested the phytochemical constituents present in them.

For the isolation of compounds, we were using chromatographic techniques by developing various solvent systems and confirm the active compounds present in them by different spectral studies.

6.2. Purification of Solvents:

Ethanol

Method I: A dry round bottom flask was fitted with a double surface condenser and a calcium chloride guard tube. Dry magnesium turnings (5mg) and iodine (0.5 gm) were placed in the flask followed by 50-75ml of commercial absolute alcohol. The mixture was warmed until the magnesium is converted to ethanolate, then 900ml of commercial absolute alcohol was added and refluxed for 30 minutes. The ethanol is distilled into vessel and used.

Method II: rectified spirit was soaked in slaked lime for over night distilled and used.

Hexane

For the purification of the hexane, it was refluxed for 4 hrs and distilled. The distilled was shaken with sufficient amount of anhydrous potassium carbonate, filtered and re-distilled. The fraction boiling at 68°C was collected and used.

Chloroform

The chloroform was shaken well with equal volume of distilled water twice to remove water-soluble impurities and separated by using separating funnel. It was then dried over anhydrous calcium chloride for 24 hours, filtered and dried once again with anhydrous potassium carbonate for another 24 hours. This was decanted and distilled; the fraction boiling at 64°C was collected and stored in an amber colored bottle.

Distilled Water

Water obtained by distillation is used for aqueous extraction of powdered drug material.

Ethyl acetate

It was refluxed for 4 hours and distilled. The distillate was shaken with sufficient amount of anhydrous potassium carbonate, filtered and re-distilled. The fraction boiling at 77°C was collected and used.[68]

6.3. Preparation of Extracts:

Preparation of extracts of *Butea monosperma (L) Toub* powdered leaves by using following solvents:

(a) Ethanol

(b) Distilled Water

(c) Hexane

(d) Chloroform

(e) Ethyl acetate

Ethanol extract

About 250gm of dried leaf powder of each of the species was taken separately in a Soxhlet apparatus and extracted exhaustively with 750 ml of 70% ethanol until the color of siphon changes, the extracts was filtered, cooled and concentrated under pressure in a rotary evaporator to a syrupy consistency followed by dried using a freeze dryer, the percentage yields of each plant are given in table no. 4. All the extracts were stored in air-tight container until further use.

Aqueous extract

The shade dried coarse powder of the leaves (250gm) was packed well in Soxhlet apparatus and was subjected to continuous hot extraction with distilled water until the completion of extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the distilled water completely. It was finally dried and kept in a desiccators till experimentation. Obtained extract was weighed and percentage yield was calculated in terms of air-dried powdered crude material.

The yield and % yield of both ethanolic and aqueous extracts of powdered leaves *Butea monosperma (L) Toub* were reported

Hexane extract:

The shade dried coarse powder of whole plant (250 gm) was packed well in Soxhlet apparatus and was subjected to continuous hot extraction with hexane for 18 hours. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. It was dried and kept in a desiccators till experimentation. Obtained extract was weighed and % yield was calculated in terms of air-dried powdered crude material.

Chloroform extract:

The shade dried coarse powder of whole plant (250 gm) was packed well in Soxhlet apparatus and was subjected to continuous hot extraction with chloroform for 18 hours. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. It was dried and kept in a desiccators till experimentation. Obtained extract was weighed and % yield was calculated in terms of air-dried powdered crude material

Ethyl acetate extract:

The shade dried coarse powder of whole plant (250 gm) was packed well in Soxhlet apparatus and was subjected to continuous hot extraction with ethyl acetate 18 hours. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. It was dried and desiccators till experimentation. Obtained extract was weighed and % yield was calculated in terms of air-dried powdered crude material. [64]

TABLE NO. 4

EXTRACTIVE VALUES OF EXTRACTS OF POWDERED LEAVES OF *BUTEA MONOSPERMA (L) TOUB.*

S. No	Extracts	Yield (in gm)	Yield % (W/W)
1.	Hexane	4.17	1.67
2.	Chloroform	7.95	3.18
3.	Ethanol	9.36	3.75
4.	Ethyl acetate	10.5	4.20
5.	water	5.93	2.37

6.4 Qualitative Phytochemical Analysis: [64,65,69]

All the extracts obtained by the powdered leaves of *Butea Monosperma (L) Toub.* were subjected to various qualitative tests for the identification of phytoconstituents present.

Test for Alkaloids:

(a) **Dragendorff's test**: To the 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) **Mayer's test:** To the 1 ml of extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

(c) **Hager's test:** To 1 ml of extract add 3ml of Hager's reagent (saturated aqueous solution of picric acid) yellow colored precipitate indicates the presence of alkaloids.

(d) **Wagner's test:** To the 1 ml of extract add 2 ml of Wagner's reagent (iodine in potassium iodide) formation of reddish brown precipitate indicates the presence of alkaloids.

Test for Saponins:

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. No layer of foam indicates the absence of saponins.

Test for Glycosides:

(a) **Legal's test:** Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. No formation of pink to red colour shows absence of glycosides.

(b) **Baljet's test:** To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange color reveals the presence of glycosides.

(c) **Keller-Killiani test:** 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer

was separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) **Borntrager's test:** Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer is treated with 1ml of ammonia. The formation of red color of the ammonical layer shows the presence of anthraquinone glycosides.

Test for Carbohydrates:

(a) **Molisch's test:** To 2ml of the extract, add 1ml of α-naphthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence.

(b) **Fehling's test:** To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

(c) **Benedict's test:** To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

Test for Tannins:

(a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

(b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.

(c) The little quantity of the extract is treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins.

(d) To the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and phenolic compounds.

Test for Flavonoids:

(a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light; formation of fluorescence indicates the presence of flavonoids.

(b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappears on addition of an acid indicates the presence of flavonoids.

(c) **Shinoda's Test**: The alcoholic extract is treated with magnesium foil and concentrated HCl give intense cherry red color indicates the presence of flavonones or orange red color indicates the presence of flavonols.

(d) The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.

(e) The extract is treated with concentrated H_2SO_4, formation of yellow or orange color indicates flavones.

(f) The alcoholic and aqueous extract is treated with 10% sodium chloride; formation of yellow color indicates the presence of coumarins.

Test for Steroids:

(a) **Libermann-Burchard test:** 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green color shows the presence of sterols.

(b) **Salkowski test:** Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

Test for Proteins:

(a) **Biuret test:** Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

(b) **Ninhydrin test:** Add two drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract

solution and heat. Development of blue color reveals the presence of proteins, peptides or amino acids.

(c) **Xanthoproteic test:** To 1ml of the extract, add 1ml of concentrated nitric acid. A white precipitate is formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange color indicates the presence of aromatic amino acids.

(d) **Millon's test:** 1ml of test solution is made acidify with sulphuric acid and add Millon's reagent and boil this solution. A yellow precipitate is formed indicates the presence of protein.

Test for Terpenes:

(a) **Noller's test:** Dissolve two or three granules of tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink color indicates the presence of triterpenoids.

Test for Gums:

Add about 10ml of aqueous extract slowly to 25ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

TABLE NO.5

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ETHANOLIC AND AQUEOUS EXTRACTS OF POWDERED LEAVES OF *BUTEA MONOSPERMA (L) TOUB.*

Phytoconstituents	**Hexane extract**	**Ethyl acetate**	**Chloroform extract**	**Ethanolic extract**	**Aqueous extract**
Alkaloids	Absent	Absent	Present	Present	Present
Saponins	Absent	Absent	Absent	Absent	Absent
Glycosides	Absent	Absent	Absent	Absent	Absent
Carbohydrates	Present	Present	Present	Present	Present
Tannins	Absent	Absent	Absent	Present	Present
Flavonoids	Absent	Present	Absent	Present	Present
Steroids	Present	Absent	Absent	Present	Absent
Proteins	Present	Absent	Absent	Present	Present
Terpenoids	Present	Present	Absent	Absent	Absent
Fats	Present	Absent	Absent	Absent	Absent

6.5. Chromatographic studies:

Chromatography is a technique for separation of the compounds of mixtures by their continuous distribution between two phase, one of which is moving past the other. The main principle of the separation may be either partition or adsorption. The aqueous and ethanolic extracts of various individual plants and its formulation were subjected to Thin Layer and High Performance Thin Layer Chromatographic studies for the separation and identification of their components.

6.5.1 Thin Layer Chromatography (TLC):

Thin layer chromatography is an important analytical tool in the separation, identification and estimation of different components. Here, when we spot a mixture of components on a TLC plates, the compounds, which are readily soluble but not strongly adsorbed moves up along with the solvent and those not so soluble but more strongly adsorbed move up less readily leading to separation of compounds.

Steps involved in TLC:

- Plate preparation with appropriate adsorbent
- Activation of adsorbent
- Sample application as spots over the chromatographic plate
- Solvent system selection
- Detecting agent/ ultra violet light
- Qualitative/Quantitative analysis

Procedure :

- Silica gel G was weighed in required quantity.
- Homogeneous slurry was made with sufficient distilled water.

- Then the slurry was poured into TLC glass plates by spreading technique and the uniform silica gel layer was adjusted to 0.25 mm thickness.
- The coated plates were allowed to dry in air and activated by heating in hot air oven at 100-105℃ for 1 hour and then used for TLC.
- The extracts were prepared with the respective solvent like ethanol and distilled water and made up to 10ml in different test tubes.
- Then with the help of capillary tube an extract was spotted on TLC plates, which were developed in TLC chamber, previously saturated with different solvent systems.[63,65,70]

By trail and error method, ethanol and aqueous extracts showed isolation and resolution of spots with following solvent systems:

1. Toluene : Ethyl acetate 7: 3
2. Petroleum ether: Ethyl acetate: Anhydrous formic acid 70:25:1

The different spots developed were identified under UV light (λ= 254 nm) and the R_f value were correspondingly calculated and tabulated as:

TABLE NO -6

TLC OF ETHANOLIC AND AQUEOUS EXTRACTS OF LEAVES OF *BUTEA MONOSPERMA (L) TOUB.*

S. NO	Extracts	Solvent Systems	No. of spots	Rf Values
1.	Ethanolic Extract	1. Toluene : Ethyl acetate 7: 3	3	0.39 0.56 0.78
2.	Aqueous Extract	2. Petroleum ether:Ethyl acetate :Anhydrous formic acid 70:25:1	2	0.19 0.32

6.5.2 High Performance Thin Layer Chromatography (HPTLC)

Introduction:

High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC) and Capillary Electrophoresis are the most widely used separation technique to establish fingerprint of the herbs, against which raw material can be evaluated and finished products can be analyzed.

High Performance Liquid Chromatography also known as planar chromatography is a modern powerful analytical technique with separation power, performance and reproducibility superior to classic TLC. Based on the use of High Performance TLC plates with small particle size (3-5 μm) and precise instruments for each step of the chromatographic process (sample application, chromatogram development, chromatogram evaluation), HPTLC provides the means not only for flexible screening procedure and qualitative analysis but also for demanding quantitative determination.

While traditional TLC often relies on visual inspection of the chromatographic plate and its documentation by either tracing or photography, HPTLC features highly sensitive scanning densitometry and video technology for rapid chromatogram evaluation and documentation. Today most HPTLC instruments are computer controlled and can therefore, offer dramatically improved reproducibility of the analytical result. At the same time HPTLC is still as flexible and friendly as classic TLC, but provides even greater advantages.

It is very useful in the qualitative and quantitative analysis of pharmaceuticals. It enjoys a practical application status, as it combines

the art of chromatography with quickness at a moderate cost. HPTLC is a major advancement of TLC principle requiring shorter time and better resolution. The basic difference between conventional TLC and HPTLC is only in particle and pore size of the adsorbants.

The plates are similar to conventional TLC plates. Layers of HPTLC plates are available in the form of precoats. Silica gel G of very fine particle size is widely used as adsorbent in HPTLC for stationary base. The use of smaller particle size helps in greater resolution and sensitivity. About 3.5 cm solvent front migration is sufficient to effect proper separation. Whatman HPTLC plates are produced from 4-5 cm silica gel with an inert binder to form a 200-cm layer.

Sample preparation in HPTLC needs a high concentrated solution, as very less amount of sample need to be applied. HPTLC is rapidly gaining importance in biochemistry of natural products and in analysis of biofluids in the field of pharamacokinetics. The analytical profiles for cardenolides, flavonoids, alkaloids, steroidal compounds etc. have been developed using the technique.

HPTLC is now days applied to obtain 'finger print' patterns of herbal formulations, quantification of active ingredients and also detection of adulteration.

Purpose of doing HPTLC:

- To perform qualitative determination and quantitative analysis of herbal extracts and synthetic drugs.
- To standardize herbal extracts & formulation
- To perform stability studies of herbal extracts
- To estimate synthetic / natural drugs in formulation

Principle:

The principle of the separation is adsorption. The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower and with lesser affinity towards the stationary phase travels faster. Thus the components are separated in a thin layer chromatographic plate based on the affinity of the component towards phase.

The different operations involved in HPTLC are as follows:

Sample Application:

Sample volume that can be applied spot wise in one stroke are 0.5 to 5 micro litres on conventional layers and 50 to 500 nano litres of HPTLC layers. Larger volumes can be applied either spot wise or sprayed in the form of narrow bands.

Narrow –band sample application ensures the highest resolution attainable with the plate/solvent combination. After all samples have been applied, the plate is developed over a short distance with a solvent of very high elution power. In this way, the rectangular sample zones are focused into narrow bands.

Chromatogram Development:

When dipping in a suitable solvent develops a thin layer chromatogram, the solvent rises in the layer by capillary action and separates the samples into their components parts. After reaching the solvent from the desired height the run is terminated.

Densitomteric Chromatogram Evaluation:

TLC Scanner is the most advanced work station for densitometric evaluation of thin layer chromatograms. It is designed for the densitometric evaluation of both TLC and electrophoresis objects. Scanning is performed in reflectance or transmittance mode, by absorbance or by fluorescence. The Scanner transmits all measurement data in digital form to the computer for further processing. Spectra recorded by the user are stored in the library. Correlation between spectra can be calculated and displayed. All methods can be run automatically.

Post Chromatographic Derivatization:

UV light is the first choice for visualization and for densitometric determination of the separated substances on the chromatogram. If the fractions do not respond to UV radiation, suitable reagents are applied to the chromatogram. For the transfer of liquid reagent, one can choose between spraying or dipping. Dipping transfer appreciably more liquid than spraying.

Procedure:

The aqueous and ethanolic (70%) extracts of *Butea monosperma (L) Toub.* K. Presl was subjected to High Performance Thin Layer Chromatography analysis in order to identify the chemical constituents.

- Camag Linomat spotted the precoated silica gel G60 of 10 X 10cm with 10 micro liter of extract.

- The plate was developed in the solvent system, Hexane: acetone: chloroform (5:3:3) for ethanolic extract and Acetone: water: acetic acid (5:5:0.5) for aqueous extract in Camag twin through chamber.
- The developed plates were taken out, dried and were scanned by Camag TLC Scanner.
- Detection was done under UV light spots of fluorescent compounds, at 254 nm.[71,72]

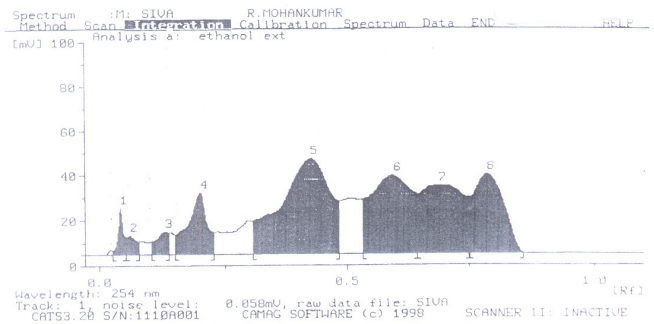
TABLE NO. 7

DATA FOR HPTLC OF AQUEOUS AND ETHANOLIC EXTRACTS OF LEAVES OF *BUTEA MONOSPERMA (L) TOUB.*

Extracts	**No. of peaks**	**R_f values**	**% Area**
Ethanolic Extract	8	0.03, 0.05, 0.12, 0.19, 0.42, 0.59, 0.69, 0.78.	1.58, 1.22, 1.77, 7.83, 31.50, 21.66, 18.84, 15.59.
Aqueous Extract	9	0.04, 0.16, 0.21, 0.45, 0.62, 0.75, 0.82, 0.86, 0.92.	6.20, 23.68, 12.78, 7.84, 10.71, 12.78, 6.75, 13.40, 5.89.

GRAPH NO. 6.1

HPTLC GRAPH OF ETHANOLIC EXTRECT OF POWDERED LEAVES OF. *BUTEA MONOSPERMA (L) TOUB.*



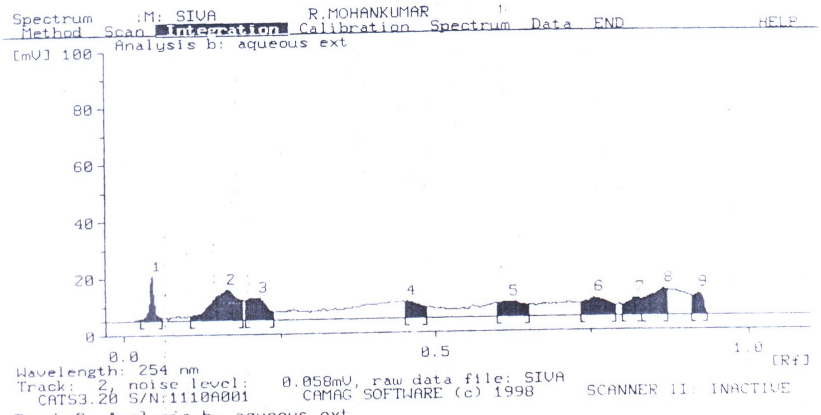
Track 1, Analysis a: ethanol ext

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.02	1.8	0.03	20.5	9.87	0.04	7.8	196.2	1.58
2	0.04	7.8	0.05	8.3	3.98	0.07	5.7	151.1	1.22
3	0.09	5.6	0.12	9.8	4.74	0.13	9.6	219.3	1.77
4	0.14	8.8	0.19	27.2	13.08	0.22	9.6	970.1	7.83
5	0.30	14.8	0.42	42.4	20.38	0.48	23.4	3902.0	31.50
6	0.53	24.0	0.59	34.4	16.56	0.64	26.1	2683.3	21.66
7	0.64	26.1	0.69	30.2	14.53	0.75	24.9	2334.2	18.84
8	0.75	25.0	0.78	35.0	16.86	0.86	0.0	1931.1	15.59

Total height = 207.8 total area = 12387.2

GRAPH NO. 6.2

HPTLC GRAPH OF AQUEOUS EXTRECT OF POWDERED LEAVES OF. *BUTEA MONOSPERMA (L) TOUB.*



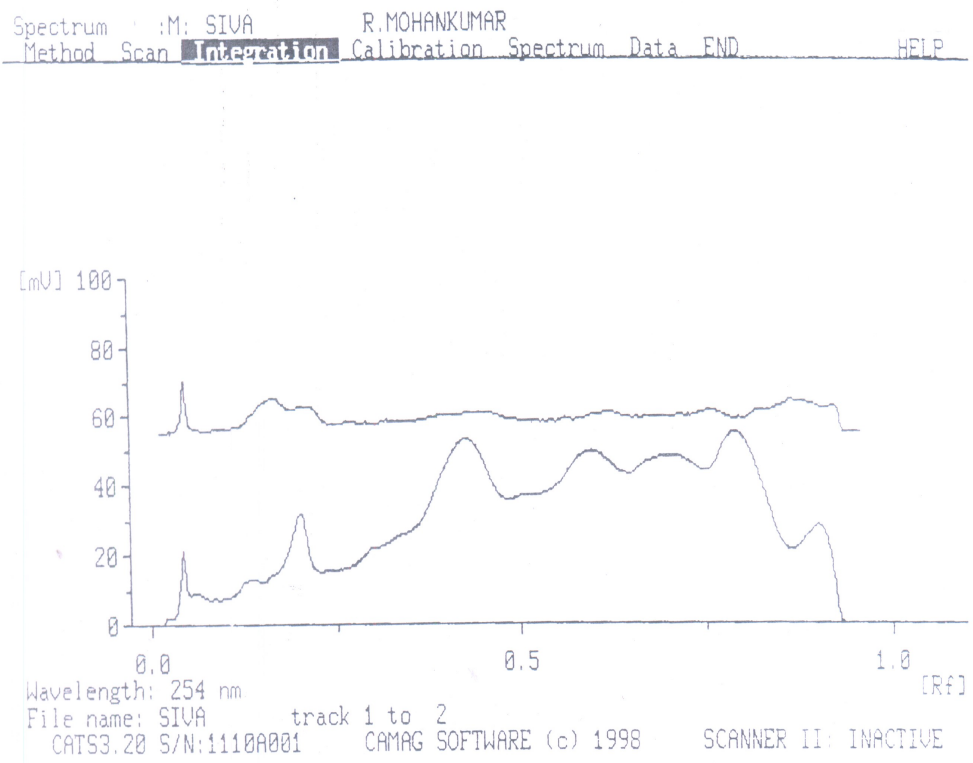
Track 2, Analysis b: aqueous ext

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.02	0.6	0.04	15.7	20.64	0.05	1.4	116.7	6.20
2	0.10	1.5	0.16	10.8	14.16	0.18	7.3	445.8	23.68
3	0.18	7.2	0.21	8.0	10.51	0.23	3.0	240.3	12.76
4	0.45	5.9	0.45	6.1	8.02	0.48	3.9	147.8	7.84
5	0.60	4.9	0.62	5.5	7.28	0.65	4.0	201.3	10.71
6	0.73	4.7	0.75	6.5	8.54	0.79	3.7	240.5	12.76
7	0.80	3.8	0.82	6.4	8.33	0.83	6.0	127.2	6.75
8	0.83	6.0	0.86	9.5	12.41	0.87	9.0	252.4	13.40
9	0.91	6.6	0.92	7.7	10.11	0.93	0.0	110.9	5.89

Total height = 76.3 total area = 1883.7

GRAPH NO. 6.3

GRAPHICAL REPRESENTATION OF HPTLC OF ETHANOLIC AND AQUEOUS EXTRACT OF POWDERED LEAVES OF. *BUTEA MONOSPERMA (L) TOUB.*



7. PHARMACOLOGICAL STUDIES

7.1 Acute Oral Toxicity study:

By following OECD (Organization of Economic Co-operation and Development) guidelines 420- **Fixed Dose Procedure (FDP)**, acute oral toxicity was evaluated. This involves the identification/calculation of the doses level that becomes evidence of non-lethal toxicity (termed Evident toxicity), which gives clear signs and symptoms of toxicity of a test drug/substance. When dose where increase to next level of highest fixed dose, which would result in the development of severe toxicity sign or even death. Next highest fixed dose producing, Evident toxicity was assumed and was also calculated on ones experiences. These doses also provide information that lead to a similar classification, to that based on the LD_{50} value.

Procedure:

- Five animals (Wister Albino rats, 150-200 gm) were selected for studies.
- Then the defined or fixed dose levels of Ethanolic and Aqueous extracts in 50, 100, 200, 500 and 1000 mg/kg was given to identify a dose producing evident toxicity.
- After giving different doses, the toxicity signs were observed within 48 hrs.
- Food was withheld for 3-4 hours after drug administration.
- Further, last highest fixed dose 2000mg/kg body weight was given and again sign of toxicity and mortality was observed.

- Most of the crude extracts possess LD_{50} value more than 2000 mg/kg of the body weight of animal used.
- Dose volume administered was 0.1ml/100gm body weight of the animal orally.
- Following was observed; body weights of the rats before and after drug administration, onset of toxicity and sign of toxicity like change in skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous system and somatomotor activity, behavior pattern, sign of tremors, convulsions, salvation, lethargy, sleep and coma was also noted if any.[73,74]

Observation:

No toxicity or death was observed for these given dose levels, in the selected and treated animals. So the LD_{50} of the Ethanolic and Aqueous extracts as per OECD guidelines-420 is greater than 2000mg/kg (LD_{50}>2000mg/kg). Hence the biological dose was fixed 100 and 200mg/kg for both the extracts.

7.2 Antidiabetic activity:

Evaluation of herbal drugs and its therapeutic use involves numerous steps i.e. starting from the proper selection and identification of plants, collection, processing, and extraction with different solvents, preliminary phytochemical studies and evaluation of therapeutic efficacy on suitable animal model and also its acute toxicity and effective dose studies.

Thus, pharmacological studies are of much importance and only the way for evaluating any herbs/drug efficacy, therefore, considering its

importance we have planned here, stepwise evaluation of antidiabetic potential of ethanolic and aqueous extracts of *Butea monosperma (L) Toub* including biochemical parameters and its antipyretic potentials as claimed in ethnobotanical studies.

Material required:

Experimental models

Male Wister albino rats of 150-200gm were selected fo either sex, for studies and they were kept in a standard polypropylene cage at room temperature of 27± 2°C, relative humidity 60-70%and well ventilated, they were fed a standard rat pellet and water ad-libitum.

Animals were deprived of food initially for 16 hr but had free access to water.

Chemical used

Freshly prepared aqueous solution of Alloxan monohydrate, (SD fine chemical Pvt. Ltd. Biosar.) Glibenclamide, carboxy methyl cellulose and text extracts (ethanolic and aqueous).

Screening of Antidiabetic activity:

Induction of Diabetes:

Hyperglycemia/diabetes was induced by single intraperitonial injection of freshly prepared aqueous solution of alloxan monohydrate 150mg/kg, to overnight fasted rats. After 48 hrs of alloxan injection, the animals which did not developed hyperglycemia i.e. glucose level>200mg/dl, were rejected/replaced with new animals. Immediately after induction of diabetes, rats were classified into five groups of six rats each.

Preparation of Text extracts:

Standard drug used for treatment, glibenclamide, 5mg/kg, ethanolic and aqueous test extracts which is prepared 200mg/kg in 2%carboxy methyl cellulose (CMC) and was given orally.

Evaluation of antidiabetic effect of test extracts was done by taking six rats in each five groups as,

Group 1: served as normal control (saline).

Group 2: served as diabetic control (alloxan induced).

Group 3 received ethanolic extract, 200mg/kg orally.

Group 4: received aqueous extract, 200mg/kg orally.

Group 5: served as reference standards (Glibenclamide, 5mg/kg).

Treatment was continued for 15 consecutive days, with twice a day dose (morning and evening). Before the treatment (0 day) and at the end of 8^{th} and 15^{th} day, blood samples were collected from the tip of the tail of each rat under mild ether anesthesia in 1ml Eppendrof tubes containing 50µl of anticoagulant (heparin) and serum separated by centrifugation of blood at 4000rpm for 10mins was subjected for estimating glucose by glucose oxidase method using semi auto-analyze.[75,76] (Table no. 8)

TABLE NO-8

ANTI-DIABETIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS

OF LEAVES OF *BUTEA MONOSPERMA (L) TOUB*

Sampling time	Blood glucose (mg/dl)				
	control	Diabetic control	Sample A (Alcoholic 200mg/kg)	Sample B (Aqueous) (200mg/kg)	Glibenclamide (5mg/kg)
8th day	125.4 ± 11.2	352.4 ± 11.8	240.8 ± 11.6*	236.7 ± 12.3*	171.6 ± 12.1*
15th day	127.2 ± 8.4	351.2 ± 11.5	216.3 ± 12.2*	198.5 ± 12.1*	139.6 ± 12.2*

Values are expressed as mean ± S.E.M. (n=6)

*compared to group received standard drug ($p<0.001$)

GRAPH NO-7.1

7.3 Anti Inflammatory Activity:

Inflammation:

"Inflammation is the reaction of vascular supporting elements to injury and results in formation of protein rich exudates provided the injuries have not been so serious as to destroy the area."

It is a localized tissue response to injuries by physical or chemical agents. It comprises a serious of phenomena occurring partly in the circulatory system and partly in the tissue in varied proportion.

Carageenan induced hind paw edema:

Albino rats of either sex weighing 150-200gms were divided into six groups of six animals each. The dosages of the drugs administered to the different groups were as follows. Group1-control, group 2,3 test drugs at a dose of 200 mg/kg and group 4-indomethacin (10 mg/kg) . All the drugs were administered orally.

After one our of the administration of the drugs, dose 0.1 ml of 1%w/v carrageenan solution in normal saline was injected in the sub plantar tissue of the left hind paw of the rat and right hind paw served as the control. The paw volume of the rats were measured in the digital plethysmograph (ugo basile, italy), at the end of 0 min, 60min. and 120min., 180min. the % increase in paw edema of the treated group was compared with that of the of control and the inhibitory effect of the drugs were studied. The relative potency of the drugs under investigations was calculated based upon the percentage inhibition of the inflammation.

Discussion:

Edema which develops after carrageenan inflammation is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The edema maintained between the first and the second phase is due to kinin like substances. The second phase is said to be promoted by prostaglandin like substances. It has been reported that the second phase of edema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin.

Percentage inhibition:

$$\frac{\text{Control (\% increase in paw Volume in 3rd hour)} - \text{Test (\% increase in paw volume in 3rd hour)}}{\text{Control (\% increase in paw volume in 3rd hour}} \times 100$$

Statistical analysis:

The experimental results were expressed as the mean ± standard error of mean (SEM) and the statistical significance was evaluated by using student‘t’ test. The p- values of less than 0.001 imply significance.[77,78]

TABLE .NO.9

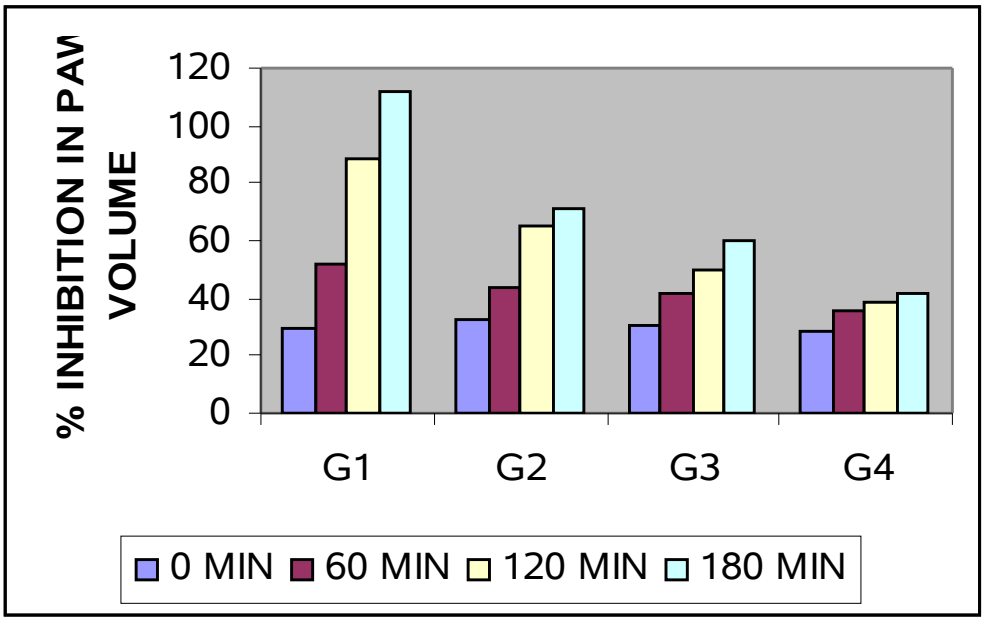
ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF LEAVES OF *BUTEA MONOSPERMA (L) TOUB* ON CARAGEENAN INDUCED HIND PAW EDEMA.

Treatment (Dose)	% increase in paw volume(Mean± S.E.M) Post result time of assay in minutes				% inhibition in paw volume
	0	60	120	180	
Control (0.1 ml/kg)	29.26 ± 1.83	51.92 ± 4.86	88.87 ± 7.05	112.01 ± 7.52	--
Ethanolic extract (100mg/kg)	32.17 ± 2.15	43.6 ± 2.69	65.2 ± 2.30	70.7 ± 3.32	51.63
Aqueous extract (200mg/kg)	30.28 ± 2.65	41.5 ± 3.21	49.6 ± 2.45	59.84* ± 4.4	46.37
Indomethacin (10 mg/kg)	28.2 ± 1.2	35.79 ± 1.82	38.6 ± 2.07	42.2 ± 3.22	65.28

* P < 0.001 Vs control by student‘t’ test.

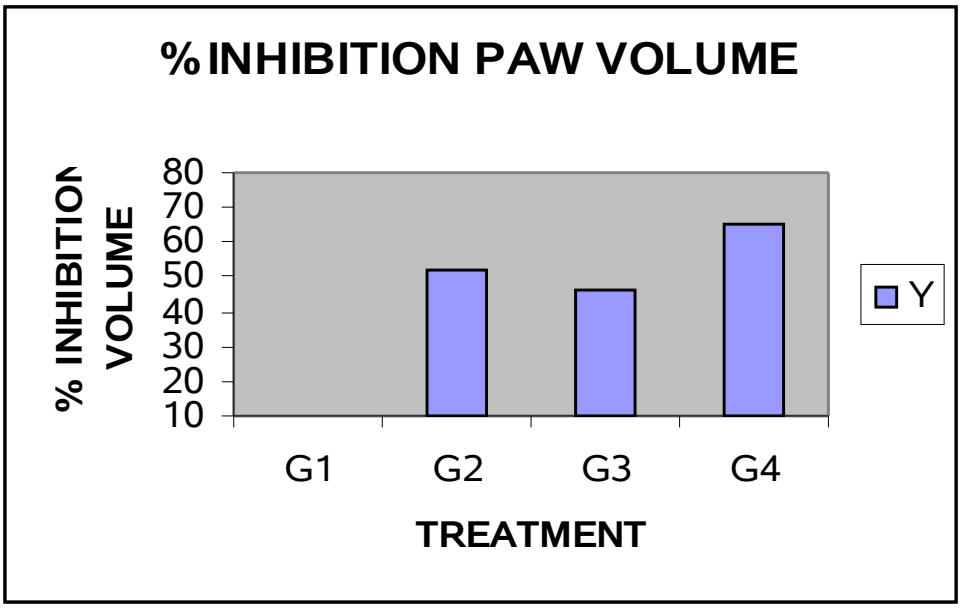
GRAPH NO-7.2

GRAPHICAL REPRESENTATION OF ANTI-INFLAMMATORY ACTIVITY OF ON ETHANOLIC AND AQUEOUS EXTRACTS OF LEAVES Of *BUTEA MONOSPERMA (L) TOUB* ON CARAGEENAN INDUCED HIND PAW EDEMA.



GRAPH NO-7.3

GRAPHICAL REPRESENTATION OF ANTI-INFLAMMATORY ACTIVITY OF ON ETHANOLIC AND AQUEOUS EXTRACTS OF LEAVES Of *BUTEA MONOSPERMA (L) TOUB* ON CARAGEENAN INDUCED HIND PAW EDEMA.



7.4 Anti-Pyretic Activity:

Introduction

Pyrexia or fever is caused as a secondary impact of infection, tissue damage inflammation, graft rejection, malignancy or other diseased status. It is the body's natural defense to create on environment where infectious agents or damaged tissue cannot survive.

The antipyretic potential of the *Butea monosperma (L) Toub* leaves studied by Brewer's yeast induced pyrexia.

Materials required:

Experimental models – Male rat weighing 200 – 250gms

Chemicals used – Aqueous suspension of dried Brewer's yeast (12%)

Screening of Antipyretic Activity:

Induction of Pyrexia

Pyrexia induced by subcutaneous injection of aqueous suspension of dried Brewer's yeast (12%) rats developing 1^{o}C or more rise in rectal temperature at 18th hour after injection were treated with 5% gm acacia.

Preparation of test extract

Standard drug used for treatment paracetomal (25mg / kg), aqueous extract (200mg/kg), ethanolic extract (200mg/kg) were prepared and given subcutaneously.

Treatment schedule

Male rat weighing 200-250 gms were divided in to four groups each consisting of six animals (n=6).

Group I : Served as control,

Group II : Received aqueous extract (200mg/kg)

Group III : Received ethanolic extract (200mg/kg)

Group IV : Served as reference standard (Paracetamol- 25mg/kg)

Treatment continued for next four hours after inducing pyrexia, temperature measured after internal of one hour up to 4th hours.

Experimental Procedure:

Male albino rat weighing 200 – 250gm were injected subcutaneously (5ml/kg) aqueous suspension of dried Brewer's Yeast (12%) Rats developing 1^{o}C or more rises in rectal temperature at 18th hr after injection were treated with 5% gum acacia and served as control. Group II & III received the

aqueous and ethanolic extract (200mg/Kg) respectively. Group IV was treated with paracetamol (25mg/Kg) and served as reference standard. Temperature was recorded at time intervals of 1, 2, 3 & 4 hours.

Percentage decrease in temperature by aqueous extract (200mg/kg), ethanolic extract (200mg/Kg) and standard drug was found to be 3.82, 4.55 and 4.80 percentages respectively.[79]

TABLE NO. 10

ANTI –PYRETIC ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *BUTEA MONOSPERMA (L) TOUB.*

S. N	Treatment	Dose Mg/Kg	Normal rectal temperature ^{O}C mean ± S.E	Temperature 18hrs after Yeast induced pyrexia	Temperature after treatments (hours)				% activi
					1st Hour ^{O}C	2nd Hour ^{O}C	3rd Hour ^{O}C	4th Hour ^{O}C	
1	Control		37.2 ± 0.05	38.09 ± 0.24	39.6 ± 0.05	39.7±0.07	39.9±0.04	39.7*±0.06	
2	The aqueous extract	200mg/ Kg	37.3 ± 0.09	39.08 ± 0.34	38.6 ± 0.24	38.8±0.12	38.5±0.09	38.01*±0.06	3.82
3	The ethanolic extract	200mg/ kg	37.6 ± 0.13	39.06 ± 0. 72	38.8 ± 0.41	38.5±0.20	38.01±0.15	37.8±0.21	4.55
4	Paracetamol	25mg/kg	37.3 ± 0.28	38.10 ± 0.16	38.5 ± 0.28	37.9 ± 0.34	37.9±0.26	37.6*±0.17	4.80

n = 6 values as mean ± S.E

*** P < 0.001 Vs control by student ‘ t ’ test**

GRAPH NO.7.4

GRAPHICAL REPRESENTATION OF ANTI-PYRETIC ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *BUTEA MONOSPERMA (L) TOUB*

8. RESULTS AND DISCUSSION

The powdered leaves of *Butea monosperma (L) Toub.* belonging to family Fabaceae has been investigated in a systematic way covering pharmacognostical, phytochemical, and pharmacological aspects in an attempt to rationalize its use as drug of therapeutic importance.

Pharmacognostical studies:

The macroscopic and microscopic details of the leaves of *Butea monosperma (L) Toub.* were investigated.

In microscopy, The leaflets are dorsiventral with prominently projecting lateral veins, thick midrib and bilateral lamina.

The midrib is very thick having adaxial, broadly conical hump and more or less wide circular abaxial part. The midrib is 1.1mm thick in vertical plane.

The lamina has even and smooths adaxial surface and uneven abaxial surface it is 170 um thick.

The vascular system is complex, it has wide hollow cylinder occupying the major area of the midrib. The vascular cylinder consists of a thick circular cylinder of sclerenchyma. The lateral veins and vein lets are thick and straight. They form narrow but distinct vein islets which are polygonal in outline and random in orientation.

The epidermal cells are narrow in area; their anticlinal walls are thin and wavy. The stomata are paracytic type with equal or unequal lateral subsidiary cells. The guard cells are elliptical or circular measuring 15x20 um in size. Epidermal trichomes are abundant on the abaxial leaf surface. The trichomes are unicellular, unbranched, narrow, and thin walled and

pointed at the tip. The trichomes are glandular type. They are up to 60um long and less than 5um thick. The cell walls are smooth.

The petiole is circular in outline, the dorsiventral, symmetry is not evident. It has thin epidermal layers of small, thick walled cells and fairly wide outer paranchymatous ground tissue. The vascular system is complex, possessing a continuous ridged outer cylinder and central excentric group of discrete strands.

The physicochemical parameters like total ash value (11.5%w/w), acid insoluble ash value (6.5%w/w), water soluble ash value (9.5%w/w) and sulphated ash value (10.4%w/w) were observed. Alcohol soluble extractive value (2.8%w/w) water soluble extractive value (1.9%w/w) and loss on drying (4.7%w/w) were evaluated The above studies enable the identification of the plant material for future investigation and forms an important aspect of drug studies.

Phytochemical studies:

The leaves of *Butea monosperma (L) Toub*. was powdered and successively extracted with hexane, chloroform, ethanol, ethyl acetate and distilled water (by continuous soxhlet extraction) the yields were 1.67 w/w, 3.18%w/w, 3.75%w/w, 4.20%w/w, 2.37%w/w respectively. All the extracts where subjected to phytochemical tests to find out the active constituents. Presence of alkaloids, terpenoids, carbohydrate, steroids, flavonoids, phenolic compounds, protein and amino acids, fat were identified.

In ethanolic extract tannins, flavonoids, steroids, alkaloids, protein, were present and in aqueous extract alkaloids, carbohydrates, tannin, flavonoids, protein were present.

Ethanoloic and Aqueous extracts were subjected to Thin Layer Chromatography on silica gel for the identification of chemical constituents. Ethanolic extract has shown three spots with R_f value 0.39, 0.56, 0.85. While aqueous extract has shown two spots with R_f value 0.19, 0.32.

Ethanoloic and Aqueous extracts were subjected to HPTLC for identifying the number of chemical constituents present in those extract. In Ethanolic extract 8 peaks were observed, in Aqueous extracts 9 peaks were observed.

Pharmacological studies:

The acute oral toxicity of ethanolic extract of the leaves of *Butea monosperma (L) Toub* was carried out as per OECD- 420 guide lines. The acute toxicity studies revealed that LD50> 2000mg/kg for the extracts.

Antidiabetic activity:

In alloxan diabetic rats the blood glucose levels were in the range of 352-358mg/kg, which was considered as sever diabetes. In the standard drug (Glibenclamide 5mg/kg) and aqueous extract (200 mg/kg) treated groups the peak values of blood sugar significantly decreased to 216.3mg/kg and 198.5 mg/kg simultaneously on the 15^{th} day. Thus the aqueous extract was found to be almost significant as standard drug in lowering blood glucose level. Where as the ethanolic extracts (200mg/kg) treated group showed 216.3 kg/mg blood glucose level which is comparatively less to aqueous extract and standard drug. The drug/extracts were given twice a day dose was found significant.

Anti-inflammatory activity:

The ethanolic and aqueous extracts both possess anti-inflammatory activity but the extent of activity was different for different extracts and also varied with doses. The ethanolic extracts (200mg/kg) possessed significant anti inflammatory activity as the percentage inhibition was 51.63(*$p<0.001$).

Anti-pyretic activity:

It was performed by using the dried Brewer's Yeast (12%) for induction of pyrexia and measuring the rectal temperature of male rat. The standard drug was served as paracetamol. The aqueous extract (200mg/kg), ethanolic extract (200mg/kg) were selected for the anti-pyretic study. The results were interpreted by measuring the decrease in rectal temperature after given test extracts and standard drug.

Decrease in temperature is observed at 1^{st}, 2^{nd}, $3^{rd,}$ & 4^{th} hour after the treatment with the extract and standard drug tabulated in Table no.10.

The treated group with leaves of *Butea monosperma (L) Toub*. both extract showed significant percentage reduction in pyrexia (3.82%, 4.55%) at 200mg/kg dose while standard drug (25 mg/kg) showed reduction in pyrexia (4.80%) .

9. CONCLUSION

The plant *Butea monosperma (L) Toub*. has been examined to gain an insight of its pharmacognostical, phytochemical and pharmacological behavior.

The pharmacognostical studies include macroscopical studies, microscopical studies, ash values, extractive values, and loss on drying.

The phytochemical investigation showed the presence of carbohydrates, alkaloids, tannin, protein and amino acids, fat, phytosterols, flavanoids in various extracts.

The findings were collaborated by TLC and HPTLC studies of all extracts indicate the presence of compounds.

The pharmacological studies showed that aqueous 200mg/kg extract showed significant antidiabetic activity. Surprisingly ethanolic 200mg/kg extracts has demonstrated significant anti-inflammatory and anti-pyretic activity.

Therefore these studies are needed to isolate and characterize the active principle of *Butea monosperma (L) Toub*. which can have offer antidiabetic, anti-inflammatory and anti-pyretic properties and to establish its mechanism of action.

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